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TITLE: Acute Pancreatitis as a Model to Predict Transition of Systemic Inflammation to Organ Failure in Trauma and Critical Illiness

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14. ABSTRACT
Trauma, extensive burns, bacterial infections, and acute pancreatitis (AP) are common conditions of tissue injury and immune system activation that can result in the systemic inflammatory response syndrome (SIRS). Surprisingly, about half of the patients with SIRS quickly recover, while the others develop a multiorgan dysfunction syndrome (MODS). SIRS and MODS do not occur immediately: SIRS evolves over a 4-12 hour period, while MODS evolves over 12-24 hours. Vascular leak syndrome (VLS) is a critical component of the transition from SIRS to MODS. Understanding the mechanism by which SIRS triggers VLS and progresses to MODS is critical to correctly model disease course thereby aiding in treatment of patients. In this report, we analyzed the serum samples for proteins that will help to understand a mechanism for cytotoxicity to endothelial cells. The results demonstrate elevated cytokine and Ang-2 levels in serum samples from patients with severe AP. Also, initial mass spectrometry findings show potential biomarkers that will be explored.

15. SUBJECT TERMS
Pancreatitis, systemic, inflammation, vascular leak, multiple organ dysfunction, biomarkers, endothelium, viability

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The problem being addressed is the unknown mechanism(s) in patients with acute pancreatitis, multiple trauma, severe burn, or sepsis responsible for the unpredictable progression of systemic inflammation to the vascular leak syndrome (VLS), which in turn leads to multi-organ dysfunction syndrome (MODS). Our experimental approach is designed to understand and predict progression from systemic inflammation to MODS. The primary observation is that serum or plasma from patients with severe acute pancreatitis (AP) or trauma with VLS is toxic to endothelial cells.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Pancreatitis, systemic, inflammation, vascular leak, multiple organ dysfunction, biomarkers, endothelium, viability

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. Define the clinical setting in which SIRS progresses, and fails to progress, to VLS and MODS using molecular and clinical measures. (months 4-36)

Aim 2. Determine the effect of serum from patients with SIRS ±VLS as well as Ang-2 and other target molecules (identified in Aim 3) on human organ-derived endothelial cells in terms of morphology, gene activation, and mode of cell death. (months 4-36)

Aim 3. Identify serum molecule(s) that best predict specific in vitro changes in endothelial cells (Aim 2) as well as which molecule(s) and endothelial cell changes best predict clinical progression to MODS (Aim 1). (months 6-36)

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

The ***first specific objective*** under **Aim 1** is to consent and enroll subjects into the study. There were 8 subjects enrolled into the study year 2. There were a total of 11 subjects in the study at the end of year 2. The ***second objective*** is to collect demographics, physiologic data, and pertinent medical information related to disease. The information from case report forms were entered into a secure database using Research Electronic Database Capture (REDCap) that can readily be converted into tabular format (Tables 1 and 2). This allows us to easily pull de-identified data compiled from case report forms and medical records for evaluation and statistical comparisons.

Table 1 & 2. Clinical Information (select)

Record ID	Age	Gender	Etiology	BMI	Temp at admission (Celcius)	Heart rate at admission (beats/min)	Resp rate at admission (breaths/min)	Pain score at admission	WBC at admission (cells/μl)
001	68	Male	Idiopathic	33.4	36.8	102	20		16,000
002	52	Female	Idiopathic	40.9	37	100	24	10	18,100
003	39	Male	Hypertriglyceridemia	37.0	36.7	95	20	10	8,200
004	68	Male	Idiopathic	36.1	37	117	16	9	26,200
005	24	Male	Hypertriglyceridemia	32.3	36.9	123	18	8	21,500
006	48	Male	Alcoholic	23.8	36.7	102	18	10	15,100
007	79	Female	Gallstones	33.3	35.1	91	21	10	15,400
008	25	Female	Gallstones	29.9	37	72	19	10	15,400
009	37	Male	Alcoholic	23.3	36.6	93	18	10	15,600

Record ID	Hematocrit (%) at admission	BUN (mg/dL) at admission	Creatinine (mg/dL) at admission	Glucose (mg/dL) at admission	AST (U/L) at admission	Pancreatic necrosis	SIRS score at admission	Ranson score at admission	Apache II score
001	48.3	38	4.1	223	457	Yes	2	3	13
002	47.9	17	0.7	362	17	Yes	3	2	5
003	47.1	9.2	0.7	384	27	Yes	1	1	2
004	49.8	24	1.6	223	57	Yes	2	3	15
005	56.9	9	0.5	165	23	No	2	1	10
006	40.6	11	0.7	100	16	Yes	2	0	3
007	48.3	37	2	241	472	No	4	3	16
008	42.8	8	0.8	124	400	No	1	1	3
009	42.1	29	0.9	122	29	No	2	0	4

Pain score – 1 – 10 (10 is worst); SIRS score – 0 to 4 depending on number of criteria met; Ranson score – 0 to 5 depending on number of criteria met; Apache II scores – 0 to 16 depending on criteria met

The ***third objective*** of Aim 1 is to analyze the serum samples for candidate toxic factors and determine if they correlate with severity. Protein measurements were performed using the Meso Scale Discovery (MSD) technology. MSD technology enables measurement of biomarker levels using electrochemiluminescense detection. This process is initiated at carbon electrodes located in the bottom of the microplates. Biological reagents can be attached to the carbon simply by passive adsorption and retain a high level of biological activity. Light at 620 nm is emitted by labels following electrochemical stimulation. Multiple excitation cycles amplify the signal to enhance detection and improve sensitivity. Cytokines well-known to be involved in the SIRS early in AP were measured including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), Tumor Necrosis Factor-α (TNF-α), chemokine Monocyte Chemotactic Protein-1 (MCP-1), and the protein angiopoietin 2 (Ang-2). Briefly, serum samples and appropriate reagents were added to 96-well plates. Protein concentrations were measured by a MSD Sector Imager 6000. All samples were analyzed in duplicates with a maximum tolerated coefficient of variation of 20%**.** Table 3 shows the specific protein concentrations in each sample. The values that are colored red indicate amounts outside of the normal ranges for each protein.

In addition to the analysis of cytokines, chemokines, and Ang-2, activin was analyzed in acute pancreatitis and matching control samples in the lab of Dr. Barbara Jung at University of Illinois Chicago. Activin is a cytokine that is a member of the TGF-β family. After ligand binding to its

type II receptors (AVCR2A or ACVR2B) activin type I receptors (ACVR1 or ACVR1B) are activated through dimerization and phosphorylation, which subsequently leads to the activation of canonical SMAD- dependent and non-canonical SMAD-independent pathways. In inflammation, activin has been reported to have both pro- and anti-inflammatory functions ex vivo, resulting in either up or down regulation of a number of key inflammatory cytokines, including IL-6, IL-1β, or IL-10 in various human and murine cell types. In vivo, activin's reported actions are primarily pro-inflammatory. It increases very early in the inflammatory response and plays a central role in such diverse inflammatory conditions as IBD, asthma and viral infections. Activin concentrations were measured in a total of 30 cases with 10 cases of mild, moderate and severe pancreatitis respectively (as per revised Atlanta criteria) and 30 healthy controls. The mild and moderate cases were

Table 3. MSD Analyses of Cytokines/Chemokines and Angiopoietin 2

Record ID	Sample ID	Day of Pain	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)	MCP-1 (pg/ml)	Ang-2 (pg/ml)
DOD001	DD900	1	19.57	4,938.88	8,217.97	8,217.97	28.37	9,558.39
DOD001	DD800	2	6.08	4,777.34	1,913.78	1,913.78	14.32	3,668.20
DOD001	DD1000	3	2.72	1,807.01	691.87	691.87	6.47	3,470.63
DOD001	DD600	4	0.61	258.52	105.43	105.43	5.16	1,951.39
DOD001	DD700	5	0.64	142.79	75.49	75.49	3.85	1,661.93
DOD001	DD500	6	0.67	451.95	78.21	78.21	4.14	1,811.51
DOD002	DD2000	1	0.29	24.75	28.48	28.48	2.38	410.56
DOD002	DD1900	2	0.20	15.01	19.42	19.42	1.85	318.60
DOD002	DD1800	3	0.25	5.53	38.72	38.72	2.13	259.44
DOD002	DD1700	4	0.12	4.49	20.86	20.86	2.56	471.60
DOD003	DD1500	1	0.72	76.18	43.63	43.63	1.75	1,100.93
DOD003	DD1400	2	0.92	103.98	27.98	27.98	1.38	1,025.82
DOD003	DD1300	3	0.57	35.37	33.92	33.92	2.15	540.97
DOD003	DD1200	4	1.14	81.06	102.06	102.06	3.39	710.20
DOD003	DD3200	5	1.21	69.87	98.40	98.40	3.12	766.57
DOD003	DD2200	6	0.97	73.29	81.56	81.56	3.20	541.84
DOD004	DD2400	1	2.24	148.26	62.56	62.56	2.32	702.49
DOD004	DD2300	2	1.30	231.22	61.27	61.27	2.53	656.86
DOD004	DD3000	4	0.94	104.21	35.02	35.02	2.27	510.11
DOD004	DD2700	5	0.69	54.11	41.14	41.14	2.45	515.40
DOD004	DD2800	7	1.25	44.41	43.03	43.03	2.77	460.93
DOD005	DD3300	3	0.42	9.41	5.85	5.85	0.99	NaN
DOD005	DD3700	4	0.36	7.01	6.39	6.39	1.96	229.87
DOD005	DD3800	5	0.44	8.07	5.88	5.88	2.72	309.56
DOD005	DD3900	7	0.22	2.76	9.86	9.86	3.74	266.30
DOD006	DD300	1	0.38	16.27	10.85	10.85	1.92	420.46
DOD006	DD4300	2	0.54	56.23	13.90	13.90	1.56	323.53
DOD006	DD3600	3	0.50	180.51	37.52	37.52	2.08	456.64
DOD006	DD3400	4	3.12	74.84	28.17	28.17	1.97	418.94
DOD006	DD4200	5	0.18	48.76	35.16	35.16	2.67	644.44
DOD006	DD4000	6	0.63	15.95	47.15	47.15	3.28	605.84
DOD006	DD5200	7	0.66	26.33	16.21	16.21	2.17	394.66
DOD007	DD100	1	0.42	32.62	58.68	58.68	4.02	866.78
DOD007	DD3500	2	2.60	320.86	187.61	187.61	14.34	1,103.75
DOD007	DD2600	3	1.13	323.82	113.40	113.40	8.72	950.52
DOD007	DD5400	4	0.66	101.70	62.39	62.39	4.23	726.21
DOD007	DD4800	5	0.82	60.04	49.50	49.50	4.27	615.29
DOD007	DD4600	6	1.12	42.00	46.26	46.26	4.34	523.76
DOD008	DD4500	2						
DOD009	DD5700	2	0.15	59.66	13.68	13.68	1.59	563.53
DOD009	DD5000	3	0.16	37.09	8.06	8.06	1.92	341.74
DOD009	DD5900	4						

Normal levels reported in the literature:
IL-1β - 0.7-1.1 pg/ml
IL-6 - 1.0-4.8 pg/ml
IL-8 - 5.0-11.3 pg/ml
TNF-α – 0.6-7.1 pg/ml
MCP-1 – 5.0-10.4 pg/ml
Ang-2 – 1,075 ± 228.2 pg/ml

obtained from the retrospective cohort study - Pancreatitis-associated Risk of Organ Failure or PROOF. The severe cases are those enrolled in this study. Serum was collected as close to hospital admission as possible, and on each of two subsequent days. Activin from human samples was measured utilizing the activin A Quantikine ELISA (R&D Systems) following the manufacturer's instructions. All samples were run in duplicates after a 1:4 dilution in PBS. Overall, serum activin levels were increased in acute pancreatitis samples when compared to controls (0.965 ng/ml versus 0.462 ng/ml, $p<0.0001$) (Figure 1A). When grouped by severity, we observed an increase in moderate and severe AP, but not in mild disease ($p<0.0001$ for difference in between groups, $p<0.05$ for moderate versus controls, $p<0.0001$ for severe versus controls, mild versus controls n.s.) (figure 1). This effect was seen both in samples at admission and when comparing all samples from AP cases. Activin levels from subsequent blood draws were not statistically different from first activin measurements. Importantly, high activin levels at admission were predictive of a longer hospital stay when compared to intermediate or low activin levels (median 26 versus 8 versus 5 days, $p<0.05$, figure 2) and even more notably longer stay in the intensive care unit (ICU) (median 23 versus 0 versus 0 days, $p<0.05$, figure 1). Also, activin levels at admission displayed a good distinguishing power between mild and severe disease with an AUC of .8200 (figure 1). At the time of the second blood draw, the predictive power was even higher with an AUC of 0.8900.

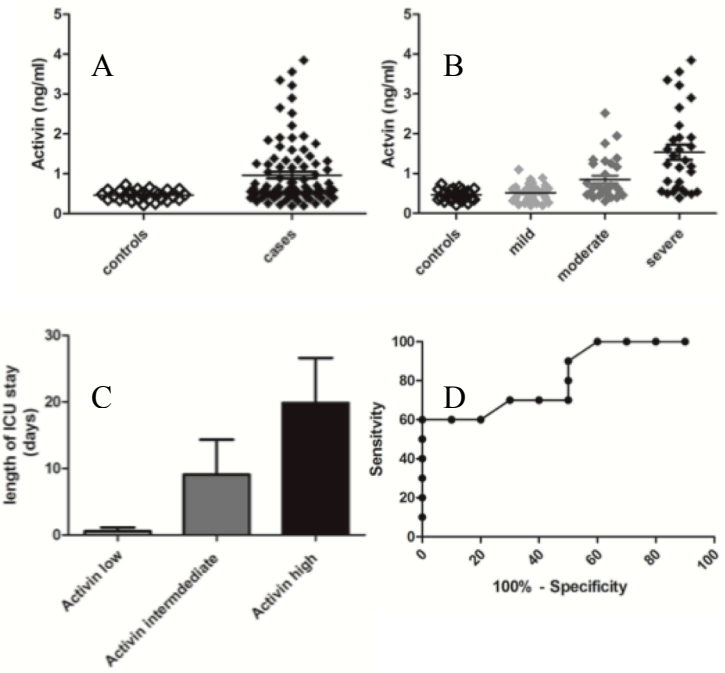


Figure 1: Activin is increased in moderate and severe AP and is correlated with worse prognosis: A) Activin is increased in samples from patients suffering from AP when compared to samples from healthy controls (upper left panel, $p<0.05$). B) Stratification by severity demonstrates that activin is specifically upregulated in moderate and severe AP, but not in mild AP (upper right panel). C) Furthermore, activin levels at admission are predictive for longer ICU stay (lower left panel). D) ROC analysis demonstrates that serum activin at admission is a good marker for distinguishing cases which subsequently develop mild or severe AP (lower right panel AUC 0.820)

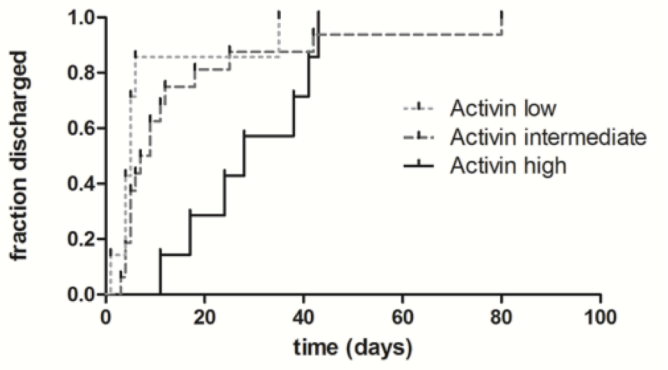


Figure 2: Activin levels at admission may be indicator of length of hospital stay: Higher activin levels were predictive of longer hospital stay according to small subset of cases.

The ***fourth objective*** was to perform proteomic analyses of the serum samples. Methods for the global determination of proteins in serum samples collected from patients diagnosed with severe acute pancreatitis enrolled in the study were being developed and tested. A serum sample from a normal healthy volunteer and subject DD002 (when screened for toxicity to the vascular endothelial cells using Molecular Probes Live/Dead Viability/Toxicity Assay (Life Technologies) showed low cell viability) were first fractionated by gel filtration chromatography on a column packed with Superdex 200 (GE Healthcare) (1.0 cm x 15 cm) using a low pressure chromatography system (Biologic LP System, Bio-Rad), UV wavelength 280 nm. The mobile phase contained 50 mM sodium phosphate and 0.15 M sodium chloride buffer, pH 7.5. This chromatography method prevents the denaturation of the proteins and allows an increased resolution of the serum protein separation leading to more robust cell culture viability experiments using the fractions collected from the chromatography column (figure 3). A more targeted mass spectrometric analysis will then be possible utilizing these "active" fractions. The peaks from the column were collected using a fraction collector and the eluted fractions dried using a Labconco CentriVap Centrifugal Concentrator. The fractions were then reconstituted in 1 ml of water and total protein in each fraction quantitated using the BCA Protein Assay (Thermo Fisher Scientific/Pierce). One μg of total protein per peak was then loaded and run on a 4-12% Bis-Tris SDS-PAGE mini-gel (Thermo Fisher Scientific) at 150V using an Invitrogen XCell SureLock Electrophoresis System to visualize the potential number of proteins in each peak. The gel was stained with SimplyBlue Safe Stain (Thermo Fisher Scientific) and specific bands of higher molecular weight excised from the gel. The samples were digested with trypsin and tryptic peptides analyzed by nano reverse phase HPLC interfaced with a mass spectrometer at the University of Pittsburgh Biomedical Mass Spectrometry Center. The tandem mass spectra (MS/MS) were analyzed by the MASCOT (Matrix Science) search engine and identified peptides and proteins further statistically validated with the Scaffold software.

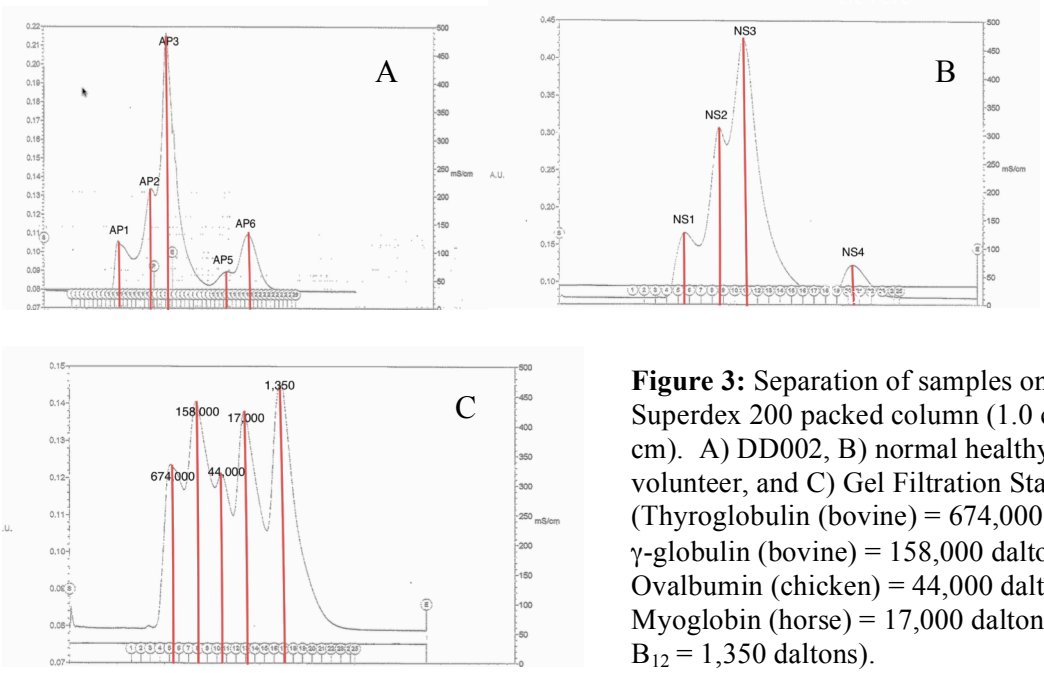


Figure 3: Separation of samples on a Superdex 200 packed column (1.0 cm x 15 cm). A) DD002, B) normal healthy volunteer, and C) Gel Filtration Standard (Thyroglobulin (bovine) = 674,000 daltons, γ-globulin (bovine) = 158,000 daltons, Ovalbumin (chicken) = 44,000 daltons, Myoglobin (horse) = 17,000 daltons, Vitamin B_{12} = 1,350 daltons).

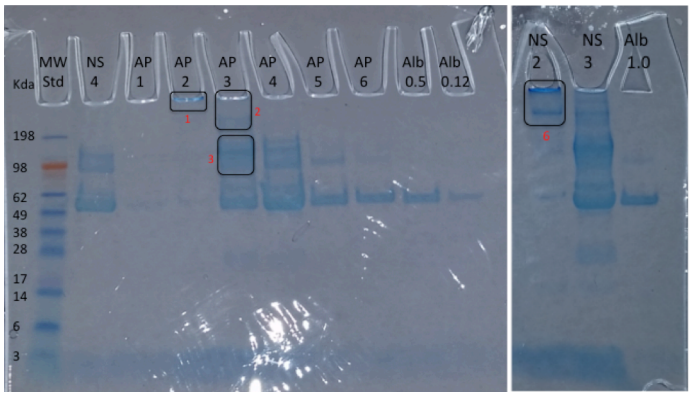


Figure 4: Separation of proteins on 4-12% Bis-Tris SDS-PAGE mini-gel showing the protein bands for the corresponding chromatography peaks. First column contains the molecular weight ladder with corresponding Kda to the left. Bands outlined with black boxes were excised for mass spectrometric analysis.

DATABASE SEARCHING-- Tandem mass spectra (MS/MS) were extracted. Charge state deconvolution and deisotoping were not performed. All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version 2.4.1). Mascot was set up to search the Uniprot_Human_BGAL_PhosB_20131212 database (unknown version, 88,505 entries) assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.80 Da and a parent ion tolerance of 1.4 Da. Carbamidomethyl of cysteine was specified in Mascot as a fixed modification. Oxidation of methionine and acetyl of the n-terminus were specified in Mascot as variable modifications. CRITERIA FOR PROTEIN IDENTIFICATION-- Scaffold (version Scaffold 4.4.8, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 90.0% probability by the Peptide Prophet algorithm (Keller, A et al Anal. Chem. 2002;74(20):5383-92). Protein identifications were accepted if they could be established at greater than 99.0% probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii, Al et al Anal. Chem. 2003;75(17):4646-58). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Some proteins identified that are unique to the SAP patient serum in gel bands 1, 2 and 3 (figure 4) are alpha-1-antichymotrypsin, ceruloplasmin, desmoglein 1, hornerin, desmoplakin, carboxypeptidase N subunit, suprabasin. In another batch of serum samples, normal, mild AP, and severe AP serum samples were fractionated and analyzed by mass spectrometry. The gel separations are shown in Figures 5 (low MWCO) and 6 (high MWCO). Bands in Figure 6 were excised and analyzed. Unique proteins to the severe AP serum were dermcidin (MW 11KDa), thioredoxin (MW 12KDa), caspase-14 (28 KDa). Proteins not

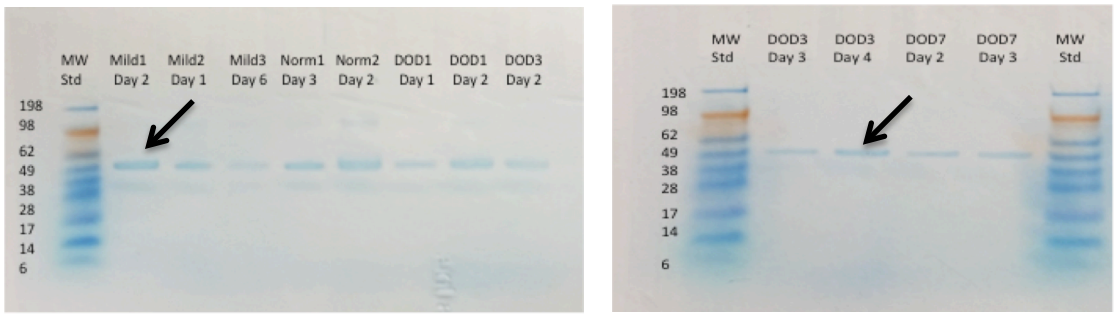


Figure 5: Separation of proteins on 4-12% Bis-Tris SDS-PAGE mini-gel showing the protein bands for the serum fractions < 100 KDa. Total amount of protein added to each lane was 1 μg. First lane contains the molecular weight ladder with corresponding KDa to the left. The bands at the approximate molecular weight of 50 KDa may correspond with albumin (66 KDa) and Ang-2 (66 KD).

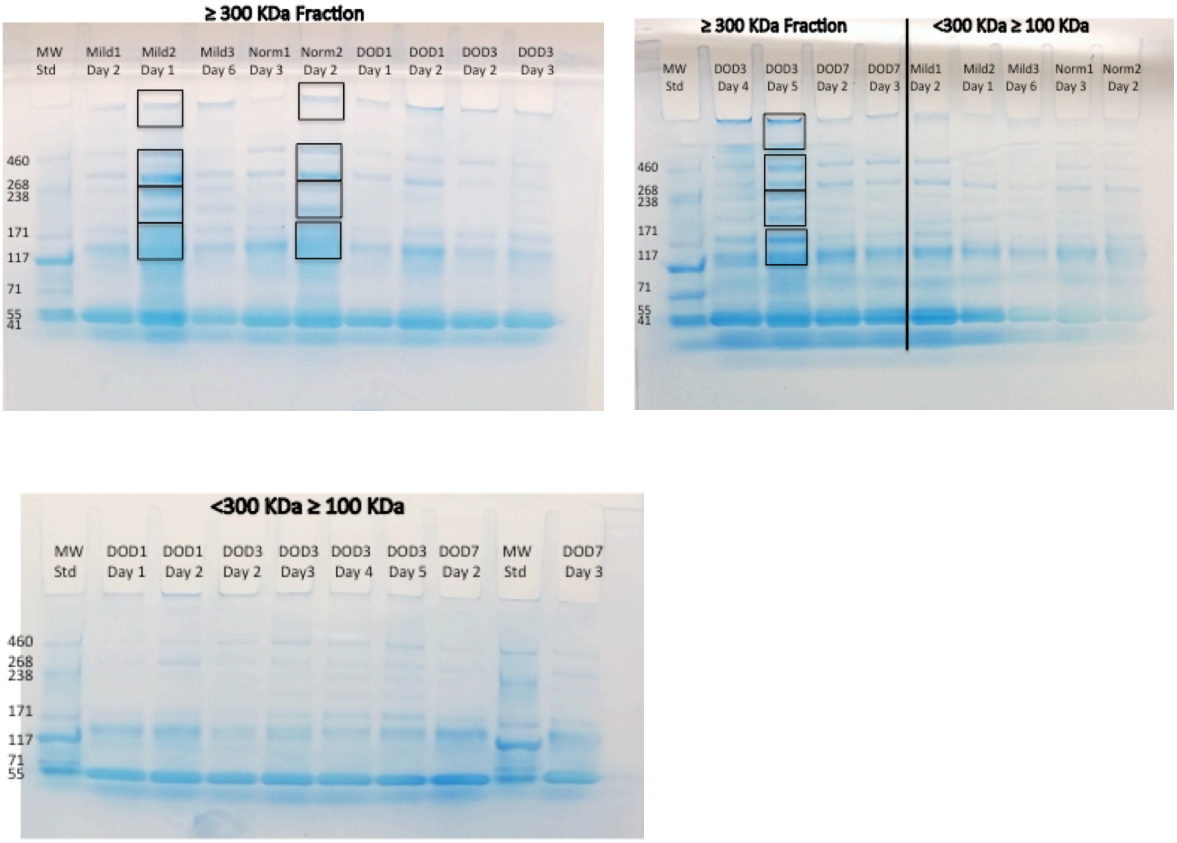


Figure 6: Separation of proteins on 3-8% Tris-Acetate SDS-PAGE mini-gel showing protein bands for MWCO ≥ 300 KDa and MWCO of 100-300 KDa. First lane contains molecular weight ladder with corresponding KDa to the left. Bands in boxes have been excised from the gels and submitted to the University of Pittsburgh Biomedical Mass Spectrometry Center for analysis.

found in the normal serum include desmoplakin (332 KDa), hornerin (282 KDa), desmoglein (114 KDa), and annexin A2 (39 KDa). These are potential biomarkers.

The specific objective under **Aim 2** was to isolate and characterize human organ-specific endothelial cells for use in the project. This will be an important ongoing process throughout the project in order to meet the goals of this study. Human intestinal microvascular endothelial cells (HIMEC) were isolated from waste surgical specimens collected directly from the operating room in order to keep the tissue viable. The cells were isolated utilizing mechanical and enzymatic processes and purified using magnetic bead selection as described previously in the January 2015 quarterly report.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Patient recruitment/enrollment into the project will be a priority. Serum will be tested for effect on endothelial cell viability. In upcoming experiments, methods to define mode of cell death (Aim 2) will be performed. The mass spectrometry findings are very interesting to date. All of the patient samples will be analyzed by mass spectrometry to determine potential biomarkers (Aim 3). Finally, recombinant Ang-2 effects on the endothelial cells will be studied and known blockers of Ang-2 will be studied to reverse the toxicity to the endothelial cells.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The recruitment of patients into the study has been slow in the second half of this year. This is due in part to the loss of our Research Nurse Coordinator in April. We are in the process of hiring a replacement. In addition, we will begin recruitment at another local, nearby UPMC branch in Shadyside. We are hopeful that enrollment will increase with the new Research Nurse Coordinator and the additional site for recruitment.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The loss of our Research Nurse Coordinator in April and Research Technician in September has lowered our expenditures of salaries. We plan to replace both of these positions in the coming year.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation);*

status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Abstracts for presentations at meetings:
Digestive Disease Week Sunday May 21, 2016: Is Endothelial Cell Injury the Link between Systemic Inflammatory Response Syndrome and Multiorgan Dysfunction Syndrome?; Annette Wilson PhD, Weiping DeBlasio RN, William Rivers BS, Efstratios Koutroumpakis MD, Georgios Papachristou MD PhD, Stephen O'Keefe MD MSc, David G Binion MD, David C Whitcomb MD, PhD; Poster
American College of Gastroenterology Tuesday, October 18, 2016: A Model to Predict Transition of Systemic Inflammation to Organ Failure in Acute Pancreatitis; Annette Wilson PhD, Weiping DeBlasio RN, William Rivers BS, Efstratios Koutroumpakis MD, Georgios Papachristou MD PhD, Stephen O'Keefe MD MSc, David G Binion MD, David C Whitcomb MD, PhD; Poster

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: David C. Whitcomb, MD
Project Role: PI
Nearest person month(s) worked: 2.4 months
Contribution to Project: Dr. Whitcomb oversaw all research in this project. Weekly research meetings were held to disseminate progress. In addition, Dr. Whitcomb wrote the IRB application and interviewed candidates for the Nurse Research Coordinator position.

Name: David G. Binion, MD
Project Role: Co-Investigator
Nearest person month(s) worked: 1.2 months
Contribution to Project: Dr. Binion provided assistance with experiments in this project and participates in research meetings. In addition, Dr. Whitcomb wrote the IRB application and interviewed candidates for the Nurse Research Coordinator position.

Name: Annette S. Wilson, PhD
Project Role: Laboratory Manager
Nearest person month(s) worked: 8.4 months
Contribution to Project: Dr. Wilson coordinated the experiments and performed imaging and data analysis. She participates in the weekly research meetings. In addition, Dr. Wilson assisted Sr. Whitcomb with writing the IRB application.

Name: Weiping DeBlasio, RN
Project Role: Research Nurse Coordinator
Nearest person month(s) worked: 2 months
Contribution to Project: Mrs. DeBlasio has consented all patients currently in the study. She has transported the blood samples to the research lab and assisted in processing, aliquotting, and storing samples. She attends the weekly research meetings. In addition, Mrs. DeBlasio coordinated renewal of the IRB proposal for this study.

Name: William M. Rivers
Project Role: Research Technician
Nearest person month(s) worked: 6 months
Contribution to Project Mr. Rivers was responsible for endothelial cell isolation, cell maintenance, and set up of experiments.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

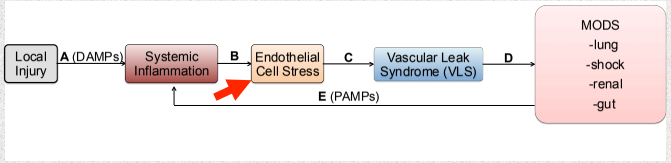
Is endothelial cell injury the link between systemic inflammatory response syndrome and multiorgan dysfunction syndrome?

Annette Wilson, Weiping DeBlasio, William Rivers, David Binion, George Papachristou, Stephen O'Keefe, David Whitcomb

Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Introduction

Trauma, extensive burns, bacterial infections, and acute pancreatitis (AP) are common conditions of tissue injury and immune system activation that can result in the systemic inflammatory response syndrome (SIRS). Surprisingly, about half of the patients with SIRS quickly recover, while others develop multiorgan dysfunction syndrome (MODS). SIRS evolves over a 4-12 hour period while MODS evolves over 12-24 hours. Vascular leak syndrome (VLS) is a critical component of the transition from SIRS to MODS. Understanding the mechanism by which SIRS triggers VLS and progresses to MODS is critical to correctly model disease course thereby aiding in treatment of patients.



Our experimental approach is designed to understand and predict progression from systemic inflammation to MODS. The primary observation is that serum from patients with severe acute pancreatitis or trauma with VLS is toxic to endothelial cells.

Severity categories following the revision of Atlanta Classification

Acute pancreatitis severity	*Organ failure and local or systemic complications*
Mild acute pancreatitis	- No organ failure - No local or systemic complications
Moderately severe acute pancreatitis	- Transient organ failure (resolves in 48 hours) - Local or systemic complications without persistent organ failure
Severe acute pancreatitis	- Persistent organ failure (single or multiple)

Methods

- Subjects diagnosed with severe acute pancreatitis (SAP) are enrolled into study.
- Inclusion/exclusion criteria are followed (see table).
- Blood was collected on days 1, 2, 3, 4, 5, 6, and 7.
- The effect of the serum on the endothelial cell viability was studied using 2 different assays – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and Molecular Probes Live/Dead Viability/Toxicity Kit. The number of live and dead cells was determined using ImageJ image analysis software (NIH) and microplate reader.
- The endothelial cells were treated with 10% of the serum samples or 1 μg/ml total protein of fractionated serum (gel filtration into fractions ≥ 100 KDa and < 100 KDa) added to the growth medium for 48 hours.

Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
- Patients 18 years of age or older - Diagnosis of acute pancreatitis (a) History of sudden onset abdominal pain (b) Elevation of amylase or lipase > 3 times normal upper clinical limits (c) Characteristic signs on abdominal imaging - Evidence of SIRS defined by 2 or more of the following features: (a) Heart rate over 90 bpm (b) Body Temp < 36 or > 38°C (c) Tachypnea > 20 breaths per minute or $PaCO_2$ < 32 mm Hg (d) WBC < 4,000 or >12,000 cell/mm^3 or > 10% immature neutrophils (bands)	- Time elapse between initial AP symptoms and collection of first serum sample > 48 hrs - Patients with chronic pancreatitis and pancreatic insufficiency based on clinical history - Pre-existing chronic renal insufficiency requiring dialysis - Pre-existing liver disease - Pre-existing immune deficiency - Pre-existing pulmonary disease - Pre-existing cardiovascular disease - Pancreatic cancer and any other forms of cancer with life expectancy < 6 months

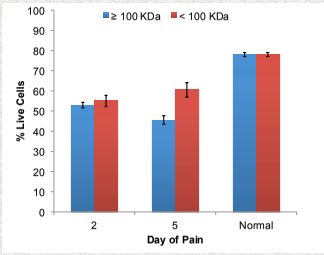
Results

Clinical data on acute pancreatitis patients

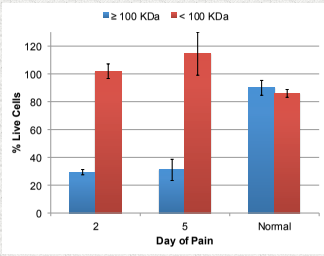
Patient no.	Age (y)	Gender	Etiology	BMI	Temp (Celsius)	HR (beats/min)	Resp rate (breaths/min)	Pain score	WBC (cells/μl)	Hct (%)	AST (U/L)	BUN (mg/dL)	Glucose (mg/dL)	Pancreatic necrosis	SIRS score	Ranson's score	Apache II score
1	68	M	Idiopathic	33.4	36.8	102	20		16,000	48.3	457	38	223	Yes	2	3	13
2	52	F	Idiopathic	40.9	37	100	24	10	18,100	47.9	17	17	362	Yes	3	2	5
3	39	M	HTG	37.0	36.7	95	20	10	8,200	47.1	27	9.2	384	Yes	1	1	2
4	68	M	Idiopathic	36.1	37	117	16	9	26,200	49.8	57	24	223	Yes	2	3	15
5	24	M	HTG	32.3	36.9	123	18	8	21,500	56.9	23	9	165	No	2	1	10
6	48	M	Alcoholism	23.8	36.7	102	18	10	15,100	40.6	16	11	100	Yes	2	0	3
7	79	F	Gallstones	33.3	35.1	91	21	10	15,400	48.3	472	37	241	No	4	3	16
8	25	F	Gallstones	29.9	37	72	19	10	15,400	42.8	400	8	124	No	1	1	3

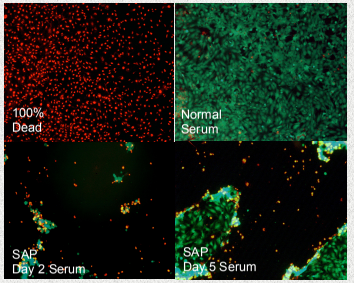
HTG: Hypertriglyceridemia; HR: Heart rate; Hct: Hematocrit; Pain score – 1 to 10 (10 is worst); SIRS score – 0 to 4 depending on number of criteria met; Ranson score – 0 to 5 depending on number of criteria met; Apache II scores – 0 to 16 depending on criteria met

Live/Dead Cell Assay - Endothelial cells treated with SAP serum fractions



MTT Assay - Endothelial cells treated with SAP serum fractions





Live/Dead Cell Assay - Endothelial cells treated with SAP serum

Conclusions

- SAP serum decreases % endothelial cell viability compared to serum from normal healthy volunteers.
- SAP serum contains proteins greater or equal to 100 KDa that decrease % endothelial cell viability.

Acknowledgements

This research was funded by the U.S. Department of Defense (Award No. W81XWH-14-1-0376).

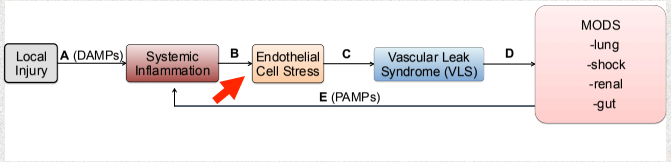
A model to predict transition of systemic inflammation to organ failure in acute pancreatitis

Annette Wilson, Weiping DeBlasio, William Rivers, Efstratios Koutroumpakis, Georgios Papachristou, Stephen O'Keefe, David G. Binion, David C. Whitcomb

Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Introduction

Trauma, extensive burns, bacterial infections, and acute pancreatitis (AP) are common conditions of tissue injury and immune system activation that can result in the systemic inflammatory response syndrome (SIRS). Surprisingly, about half of the patients with SIRS quickly recover, while others develop multiorgan dysfunction failure (MODS). SIRS evolves over a 4-12 hour period while organ failure evolves over 12-24 hours. Vascular leak syndrome (VLS) is a critical component of the transition from SIRS to MODS. Understanding the mechanism by which SIRS triggers VLS and progresses to organ failure is critical to correctly model disease course thereby aiding in treatment of patients.



Our experimental approach is designed to understand and predict progression from systemic inflammation to MODS. The primary observation is that serum from patients with severe acute pancreatitis or trauma with VLS is toxic to endothelial cells.

Severity categories following the revision of Atlanta Classification

Acute pancreatitis severity	*Organ failure and local or systemic complications*
Mild acute pancreatitis	- No organ failure - No local or systemic complications
Moderately severe acute pancreatitis	- Transient organ failure (resolves in 48 hours) - Local or systemic complications without persistent organ failure
Severe acute pancreatitis	- Persistent organ failure (single or multiple)

Methods

Demographic and phenotypic data were collected on the patients. The serum samples were characterized for markers of acute pancreatitis including lipase, amylase, specific cytokines/chemokines and angiopoietin 2 (Ang 2). We compared the effect of mild and severe acute pancreatitis patient and normal healthy control serum samples on the viability of human vascular endothelial cells grown in a monolayer using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The serum samples were then fractionated to isolate the active fraction(s) based on molecular weight. The effect of the enriched fractions on the endothelial cells was assessed using the Molecular Probes Live/DeadViability/Toxicity Kit (Life Technologies).

Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
- Patients 18 years of age or older - Diagnosis of acute pancreatitis (a) History of sudden onset abdominal pain (b) Elevation of amylase or lipase > 3 times normal upper clinical limits (c) Characteristic signs on abdominal imaging - Evidence of SIRS defined by 2 or more of the following features: (a) Heart rate over 90 bpm (b) Body Temp < 36 or > 38°C (c) Tachypnea > 20 breaths per minute or $PaCO_2$ < 32 mm Hg (d) WBC < 4,000 or >12,000 cell/mm^3 or > 10% immature neutrophils (bands)	- Time elapse between initial AP symptoms and collection of first serum sample > 48 hrs - Patients with chronic pancreatitis and pancreatic insufficiency based on clinical history - Pre-existing chronic renal insufficiency requiring dialysis - Pre-existing liver disease - Pre-existing immune deficiency - Pre-existing pulmonary disease - Pre-existing cardiovascular disease - Pancreatic cancer and any other forms of cancer with life expectancy < 6 months

Results

Clinical data on acute pancreatitis patients

Patient no.	Age (y)	Gender	Etiology	BMI	Temp (Celsius)	HR (beats/ min)	Resp rate (breaths/ min)	Pain score	WBC (cells/ µl)	Hct (%)	AST (U/L)	BUN (mg/ dL)	Glucose (mg/dL)	Pancreatic necrosis	SIRS score	Ranson's score	Apache II score
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3	39	M	HTG	37.0	36.7	95	20	10	8,200	47.1	27	9.2	384	Yes	1	1	2
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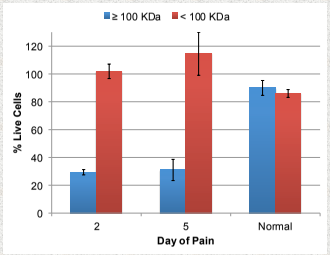
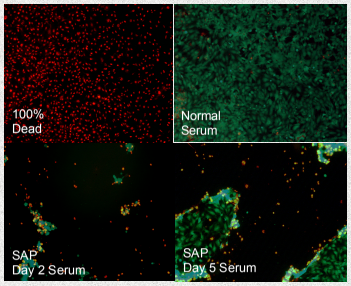
7	79	F	Gallstones	33.3	35.1	91	21	10	15,400	48.3	472	37	241	No	4	3	16
8	25	F	Gallstones	29.9	37	72	19	10	15,400	42.8	400	8	124	No	1	1	3
9	37	M	Alcoholism	23.3	36.6	93	18	10	15,600	42.1	29	6	122	No	2	0	4

HTG: Hypertriglyceridemia; HR: Heart rate; Hct: Hematocrit; Pain score – 1 to 10 (10 is worst); SIRS score – 0 to 4 depending on number of criteria met; Ranson score – 0 to 5 depending on number of criteria met; Apache II scores – 0 to 16 depending on criteria met



MTT Assay – Endothelial cells treated with SAP serum fractions



Live/Dead Cell Assay - Endothelial cells treated with SAP serum

Inflammatory marker levels in acute pancreatitis patients

Patient no.	Day no.	Amylase (U/L)	Lipase (U/L)	TG (mg/dL)	CRP (mg/dL)	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF-α (pg/mL)	MCP-1 (pg/mL)	Ang-2 (pg/mL)
1	1	230	439	N/A	N/A	19.57	4,939	8,218	28.4	9,558	217,369
1	6					0.67	452	78.2	4.14	1,812	82,558
2	1	803	822	210	31.55	0.29	24.8	28.5	2.38	411	6,840
2	4					0.12	4.49	20.9	2.56	472	18,517
3	1	N/A	798	5,242	20.7	0.72	76.2	43.6	1.75	1,001	17,402
3	6					0.97	73.3	81.6	3.20	542	25,363
4	1	N/A	557	91	35.55	2.24	148	62.6	2.32	702	11,112
4	7					1.25	44.4	43.0	2.77	461	20,208
5	3	N/A	N/A	N/A	N/A	0.42	9.41	5.85	0.99	230	7,968
5	7					0.22	2.76	9.86	3.74	266	2,955
6	1	2,548	10.073	N/A	N/A	0.38	16.3	10.9	1.92	420	3,551
6	7					0.66	26.3	16.2	2.17	395	7,244
7	1	1,325	3,190	N/A	N/A	0.42	32.6	58.7	4.02	867	22,945
7	6					1.12	42.0	46.3	4.34	524	25,026
9	2	441	4,026	46	4.048	0.15	59.7	13.7	1.59	564	3,002
9	3				15.048	0.16	37.1	8.06	1.92	342	2,913

Norm values: Ang 2 = 1,075 ± 228.2 pg/mL; IL-1β = 0.7-1.1 pg/mL; IL-6 = 1.0-4.8 pg/mL; IL-8 = 5.0-11.3 pg/mL; TNF-α = 0.6-7.1 pg/mL; MCP-1 = 5.0-104 pg/mL; Lipase = 15-70 U/L; Amylase < 65 U/L; TG < 150 mg/dL; CRP < 1 mg/dL

Conclusions

- SAP serum decreases % endothelial cell viability compared to serum from normal healthy volunteers.
- SAP serum contains proteins greater or equal to 100 KDa that decrease % endothelial cell viability.
- Ang 2 levels are extremely elevated in patients with severe acute pancreatitis.
- MCP-1, IL-6 and IL-8 are elevated in severe acute pancreatitis and remain elevated at the later time points.

Acknowledgements

This research was funded by the U.S. Department of Defense (Award No. W81XWH-14-1-0376).